pH

The pH was determined non-destructively using a Knick pH meter 765 Calimatic (electrode Mettler-Toledo 406-M6-DXX-S7/25). The pH was read at three different points of the sample. At each point, two successive readings were done. The average of the readings gives the pH value of the sample.

Moisture content

The moisture was determined by the difference in mass of the samples after and before freeze-drying for 48 h (Lyoquest, Telstar). Approximately 10 g of fresh samples were weighed into Falcon tubes covered with perforated aluminium foil. The Falcon tubes were placed on the freeze-dryer. After 24 hours, the Falcon tubes were taken out and weighed on an analytical balance. The moisture content (as a percentage) of the samples was calculated as follows:

Moisture =[(M1-M2)/M1] ×100 with M1: mass of fresh sample, M2: mass of freeze-dried sample

Crude protein determination by Kjeldahl method (ISO, 2014)

The crude protein content was determined by the Kjeldahl method. Average 0.5g of dry samples was heated at 420°C for 1 hour with sulfuric and phosphoric acids (H_2SO_4 – H_3PO_4 et H_2O_2) in the presence of catalysts kjeltabs. The total organic nitrogen is converted to ammonium sulfate. The digest is neutralized with alkali and distilled into a boric acid solution. The borate anions formed are titrated with sulfuric acid 0.2 N, which is converted to nitrogen in the sample using the formula: crude protein (% weight wet) = (V*N*0.014*100*6.38)/PE

V = volume of sulfuric acid used

N = normality of sulfuric acid used

PE = weight of test sample in grams

6.38 = nitrogen-to-protein conversion coefficient for dairy products

Fat and fatty acids (Douny et al. 2015)

Extraction of the total lipids was done using the shaken solvent extraction method with chloroform/methanol (2:1, v/v) follow by an evaporation in an oven at 60°C (Folch, 1957). Fifty milligrams of fat extracted were mixed with 5 mL hexane and 10 µL were used for the saponification/methylation of the fatty acids. Internal standard nonadecanoic acid (C19:0) was then added and hexane was evaporated to dryness under a stream of nitrogen. One milliliter toluene and 2 mL sulfuric acid 2% (v/v, in methanol) were added to the fat and the capped tube was heated in a water bath at 100 °C for 1 h, with vigorous agitation thanks to a magnetic stirrer. Then, 3 mL NaCl 5% were added and the methyl esters were extracted with two times 2 mL hexane. The extract was washed with 4 mL K2CO3 2% (w/v) and Na2SO4 was added to a part of the extract. The extract was then evaporated to dryness in a SavantTM Universal SpeedVacTM Vacuum System (Thermo Fisher Scientific, Waltham, MA) in order to eliminate the toluene. Three hundred and fifty-five microliters hexane were added and the tube was vortexed. Finally, 80 µL was transferred into an injection vial and 20 µL gadoleic acid methyl ester (C20:1-ME) was added to be used as the injection standard. For the calibration curve, the same protocol was applied to hexane solutions containing a pool of 23 fatty acids, at six different concentration levels (from 0.06 to 16.68 ng μL^{-1}). FAME were separated on a Focus GC gas chromatographer (Thermo Fisher Scientific) using a CP-Sil88 column for FAME (100 m 9 0.25 mm, 0.2 lm) (Varian; Agilent Technologies, Santa Clara, CA) and analyzed with an ion trap PolarisQ mass spectrometer (Thermo Fisher Scientific). The GC conditions were: inlet: 250°C; splitless injection; helium as the carrier gas at 1.5 mL min1; temperature program: 55°C for 1 min, followed by an increase of 5°C min1 to 180°C, then 10°C min1 to 200°C for 15 min, then an increase of 10°C min1 to 225°C for 14 min; total run time was 59.50 min. Injection volume was 1 lL. The peaks were identified by comparing their mass spectrum and retention times with those of the corresponding standards. The MS conditions were: transfer line: 250°C; ion source: 220°C; collision energy: 35 eV; positive ionization mode. The FAME were detected

using selected ion monitoring (SIM) mode in five segment windows. In each chromatographic run, different ions were monitored for each fatty acid analyzed, which allowed to perform detection and quantitative analysis: m/z 101 + 143 for saturated, 79 + 91 for mono and polyunsaturated fatty acids. The 27 FAME, the internal standard, and the injection standard were separated in a run time of 1 h using the optimized GC–MS parameters. For quantification, a 6-point calibration curve containing standard solutions and the internal standard was performed for each of the 27 fatty acids methyl esters determined. The response (ratio between fatty acids methyl esters and the internal standard peak areas) was plotted against standard concentrations. A linear regression was used and no "fit weighting" was applied.

Essential minerals and metallic trace elements (Gobert et al. 2017)

Before the analysis, samples were thawed and cleaned with ultrapure water. Samples were mineralized in Teflon digestion vessels, in a closed microwave digestion labstation (Ethos D, Milestone Inc.), using nitric acid and hydrogen peroxide as reagents (suprapur grade, Merck). Minerals and metallic trace elements were determined by Inductively Coupled Plasma Mass Spectrometry using Dynamic Reaction Cell technology (ICP-MS ELAN DRC II, PerkinElmer ®). In order to check the purity of the chemicals used, a number of chemical blanks were run; there was no evidence of any contamination in these blanks. Analytical quality control was achieved using Certified Reference Materials (CRM), DOLT-3: dogfish liver, NIST 1566b: oyster tissue, NIST 1577c: bovine liver and NIST 2976: mussel tissue. The results obtained on the Certified Reference Materials were consistent with the certified values (global mean recovery was 92 ± 16%). For each mineral and metallic trace element, detection decision (LC), detection limit (LD) and quantification limit (LQ) were calculated, depending on their specific blank distribution. The results are expressed in milligrams of element per kilogram of body dry wet (mg kg−1 dw).

Dioxins and dioxin-like PCBs (polychlorinated biphenyls) compounds (Scippo et al. 2004)

The DR-CALUX (Dioxin responsive chemically activated luciferase gene expression) was developed by Wageningen University and is distributed by BioDetection System (BDS, NL). This assay involves the rat hepatoma H4IIE cell line stably transformed with an AhR-controlled luciferase reporter gene construct. Fat of the samples was extracted by a shaken solvent extraction method with *n*-hexane/diethyl ether (97:3, v/v) (Merck, Darmstadt, Germany) and evaporated using the rotavapor (35°C, 90 rpm). The clean-up was performed on acidic silica columns and dioxins were eluted with *n*-hexane/diethyl ether (97:3, v/v) (Merck, Darmstadt, Germany). The cleaned extract containing dioxin-like compounds was further evaporated to 50 µL, under a gentle stream of nitrogen. Just prior completing evaporation of the solvent, DMSO (Across organics) was added and the remaining solvent was evaporated. The same procedure was applied to solvent instead of sample, to estimate background. DR-CALUX analysis was performed by exposing the cells (in triplicate, in 96 wells plates) during 24 h to sample extracts or to standard TCDD solutions in DMSO diluted in culture medium (-MEM, Invitrogen) containing 10% (v/v) of foetal calf serum (FCS, Invitrogen). The final concentration of DMSO in culture medium was 0.4% (v/v). After cell lysis and substrate addition (buffer containing 1% luciferin [Promega] and 0.5 mM ATP [Roche Diagnostics Belgium]), luminescence was measured using a luminometer Orion II (Berthold Detection System, Germany). DR-CALUX concentrations were calculated from a standard calibration curve, ranging from 0 (blank DMS0) to 20 pg TCDD per well, and established in triplicate on each 96 wells plate. Dose response curves were fitted using a user-defined curve fit (Slide Write Plus v. 6.1, Advanced Graphics Software, USA): $y = \frac{a0}{[1+(x/a1)\exp a2]}$ where y is the measured response (the luminescence expressed in RLU for Relative Light Unit), x is the concentration of the test compound, $a0$ is the maximal response, $a1$ is the EC50 (EC: Effective Concentration; EC50: concentration needed to reach 50% of the maximal response), $a2$ is the slope of the curve.

Aflatoxin M1 determination

AFM1 was determined by ELISA (Enzyme-Linked Immunosorbent Assay) method using the RIDASCREEN kit of R-Biopharm (Germany). Methanol (20 ml, 70%) was added to the triturated cheese sample (5 g), incubated at 50°c for 30 min (5 times shaken during incubation) and centrifugated (10 min / 3000 g / 10 ° C). 2 ml of aqueous phase was added to 2 ml of hexane, mixed, and centrifugated (10 min / 3000 g / 10 ° C). 100 µl of the lower aqueous phase was diluted with 400 µl of buffer (dilution 1:5) and 100 µl of the resulting solution was used per well in the test. A sufficient number of microtiter wells were inserted into the microwell. 100 µl of antibody solution were added and incubated at room temperature for 15 min. The liquid was poured off the wells and the microwell holder was tapped upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. All the wells were filled with washing buffer 3 times and emptied as described earlier. 100 µl standard solutions and prepared samples in separate wells were added and incubated for 30 min at room temperature in the dark. The washing procedure was repeated once. 100 µl of the enzyme conjugate was added and incubated for 15 min at room temperature in the dark. The washing sequence was repeated three times. 100 µl of substrate/chromogen were added to each well and mixed thoroughly and incubated for 15 min at room temperature in the dark. 100 µl of the stop reagent was added to each well and mixed and measured at an absorbance of 450 nm against an air.

Biogenic amines

Two grams of homogeneous fresh sample was placed into a 10 mL glass tube with 100 µl of internal standard and 2.4 mL perchloric acid 0.4 M. The mixture was shaken 15 min on a rotating shaker and centrifuged (10 min, 3700g, room temperature). The supernatant was transferred into a 15 mL Falcon tube and 2.5 mL of perchloric acid 0.4 M were added to the sample, which was extracted and centrifuged once again. Both supernatants were combined and volume was adjusted to 6 mL with perchloric acid 0.4 M. The extract was vortexed and 2 ml

was filtered through an Acrodisc filter (25 mm, 5 μm). One milliliter was then transferred in a clean 15 mL Falcon tube where 200 μL NaOH 2 N and 300 μL of saturated NaHCO3 were added, with vortex of the tube after each addition The dansylation was realized by adding 2 mL dansyl chloride (10 mg/mL in acetone) and incubating the tubes at 70 °C for 15 min in a water bath. After dansylation, 100 μL glycine (150 mg/mL in water) was added to bind to the dansyl chloride in excess, the tube was vortexed and a second incubation during 15 min at 70 °C was realized. The tube was then centrifuged (5 min, 3700g at room temperature). Finally, 1 ml of the solution was poured into an injection vial which was capped and the samples were kept at 5 °C in the autosampler until analysis. Amines were analyzed on a UPLC (Ultra-Performance Liquid Chromatography combined with Fluorescence Detection) Acquity system integrated autosampler (Acquity Sample Manager FTN), solvent delivery system (Acquity QSM H Class), and column heater coupled to an Acquity Fluorescence detector, all rom Waters Corporation (Milford, MA, USA). The column used was an Acquity UPLC BEH C18 (2.1 × 100 mm, 1.7 μm), with a UPLC BEH C18 VanGuard pre-column (2.1 × 5 mm, 1.7 μm), both from Waters Corporation. The mobile phase was water (solvent A) and acetonitrile (solvent B). The gradient elution conditions 70% solvent A were maintained for 1 min, from 70 to 15% of solvent A within 22 min, then conditions were held for 0.50 min and the contribution of solvent A was increased to 70% over 1 min and maintained for 1.50 min, with a total run time was of 26 min. The oven temperature was set at 65 °C and the injection volume was 5 μL. The flow rate was 0.4 ml/min. The peaks were identified by comparing their retention times with those of the corresponding standards. Fluorimetric detection at 346 nm for excitation and 500 nm for emission was applied. Results were calculated using Empower 3 Software (Waters Corporation).

Screening of antibiotic residues by beadyplex

BeadyPlex is a simple multiplex and multimatrix solution for the simultaneous screening of more than 80 antibiotic residues. 1 g of fresh samples was mixed with 1 ml of extraction buffer and shaken for 10 min, then centrifuged for 15 min. The supernatant obtained was filtered into 96-well microplates. 50 µl of the filtrate from each well was mixed with 50 µl of assay competitor and 50 µl of primary binders. The resulting mixture was incubated for 30 min and then washed. Next, 150 µl of secondary binders were added, incubated for 15 min and washed. Finally, 150 µl of buffer was added to each well.The plates were read by the flow cytometer ACEA NovoCyte® 2000.

Pesticide residues analysis

Cheese samples were analysed on a Waters ACQUITY UPLC™ (Ultra Performance Liquid Chromatography), equipped with a quaternary pump and membrane degasser. The separation column, an Acuity UPLC BEH C18, 130Å, 1.7 µm, 2.1 mm x 50 mm, was kept at 40°C. An automatic injector was set to inject 10 µl per sample. The mobile phase components were (A) Mille-Q water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. The gradient used was set at a flow rate of 0.4 mL min-1 of 98% mobile phase A for 0.25 min. From 0.25 min to 7 min, a linear gradient was used to 98% mobile phase B, which was maintained for 1 min. Then, a linear gradient was used to 98% mobile phase A and maintained for 1 min. Sample analyses were performed using a triple quadruple system with electro spray ionization (Waters Xevo® TQD mass spectrometer detection; Waters, Zellik, Belgium). The capillary needle was maintained at +2 kV. For operation in the MS/MS (Mass Spectrometry) mode, the following parameters were set: curtain gas (N2) at 7 bar; temperature 500°C. The active ingredients (AIs) were monitored and quantified using multiple reaction monitoring (MRM). Optimization of the MS/MS conditions, identification of the parent and product ions, as well as the selection of the cone and collision voltages, was performed through direct infusion of their individual standard

solutions. After the optimization of the collision cell energy, two different m/z transitions were selected for each analyte, one for quantification and one for confirmation.

Chlorinated pesticides were analyzed using an Agilent Technologies 6890N gas chromatograph equipped with an Agilent Technologies 7683 Series auto sampler injector, coupled to an electron capture detector (GC-ECD). Separation was performed on a HP-5MS (5% phenyl methyl siloxane) capillary column (30 m × 0.25 mm, 0.25 μm film thickness). The temperature of the injector and detector was maintained at 200°C and 250°C, respectively. Helium was used as a carrier gas at a flow rate of 1.1 mL min-1 and the injections were made in the split mode with a split ratio of 52.7:1. Concentrations of 0.004 mg/L, 0.01 mg/L, 0.02 mg/L, 0.04 mg/L and 0.1 mg/L were prepared in hexane from a stock solution for each active ingredient to form the calibration curve. The recovery analysis was conducted for each active ingredient using the spike-placebo recovery method. For this, four blank samples were spiked and analyzed under the same conditions with the same extraction procedure. As the concentration and volume of the spiked solution is known, the recovery can be calculated for each active ingredient.